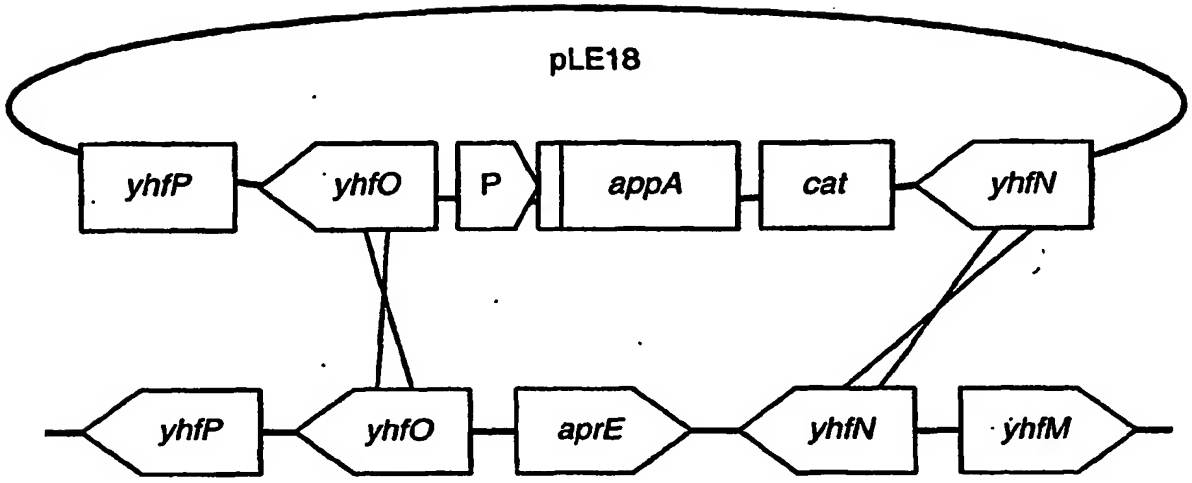

FIG._1

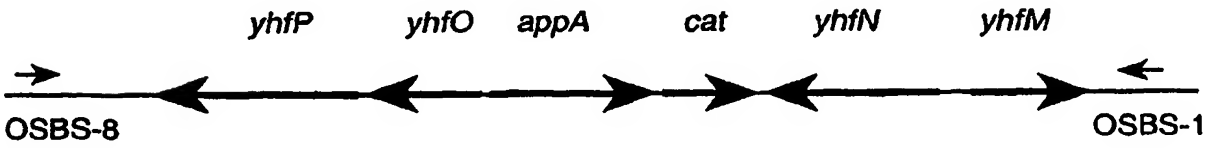

FIG._2

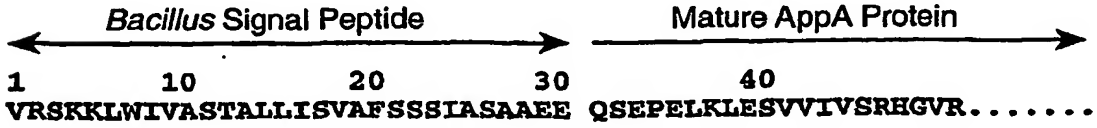

FIG._3

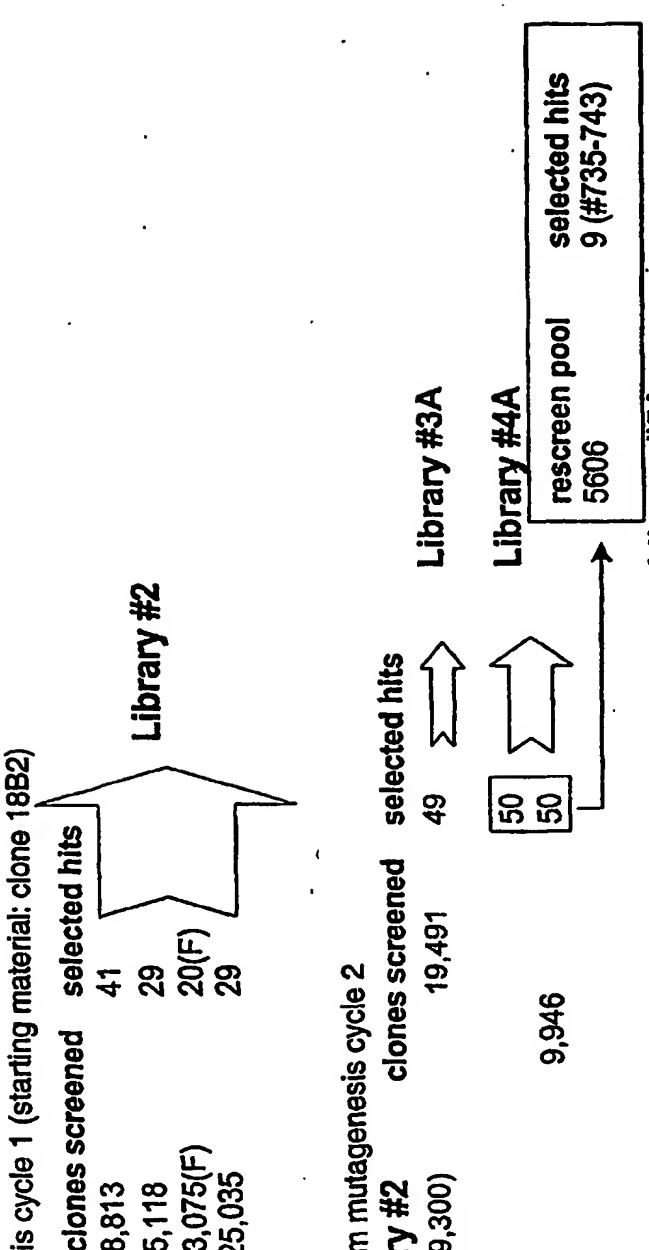
Random mutagenesis cycle 1 (starting material: clone 18B2)
Library #1
(size: 10,000)
clones screened
8,813
5,118
3,075(F)
25,035
selected hits
41
29
20(F)
29
Library #2
Random mutagenesis cycle 2
Library #2
(size:29,300)
clones screened
19,491
9,946
19,592
selected hits
49
50
50
43
Library #3A
Library #4A
rescreen pool
5606
selected hits
9 (#735-743)
Library #5A
Random mutagenesis cycle 3
Library #3A
(size:20,000)
clones screened
8,488
selected hits
50 (#635-#684)
Library #4A
(size:30,000)
clones screened
21,564
21,100
selected hits
50 (#685-#734)
50 (#744-#793)
rescreen pool
2134
selected hits
15 (#844-#858)
Library #5A
(size:100,000)
clones screened
21,763
30,921
selected hits
50 (#794-#843)
50 (#859-#908)

FIG._4

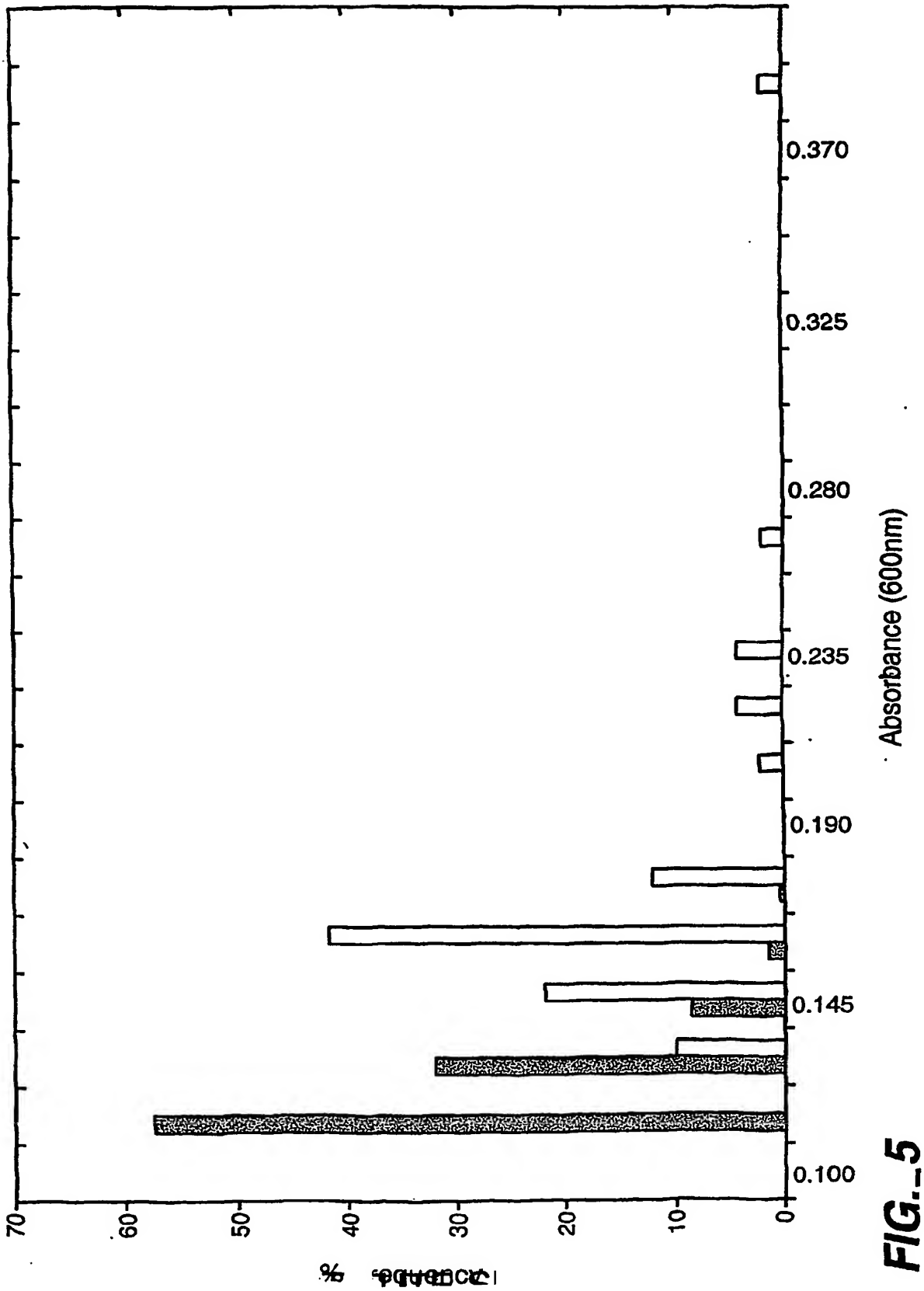
0.370
0.325
0.280
0.235
0.190
0.145
0.100
Absorbance (600nm)
70
60
50
40
30
20
10
0

FIG._5

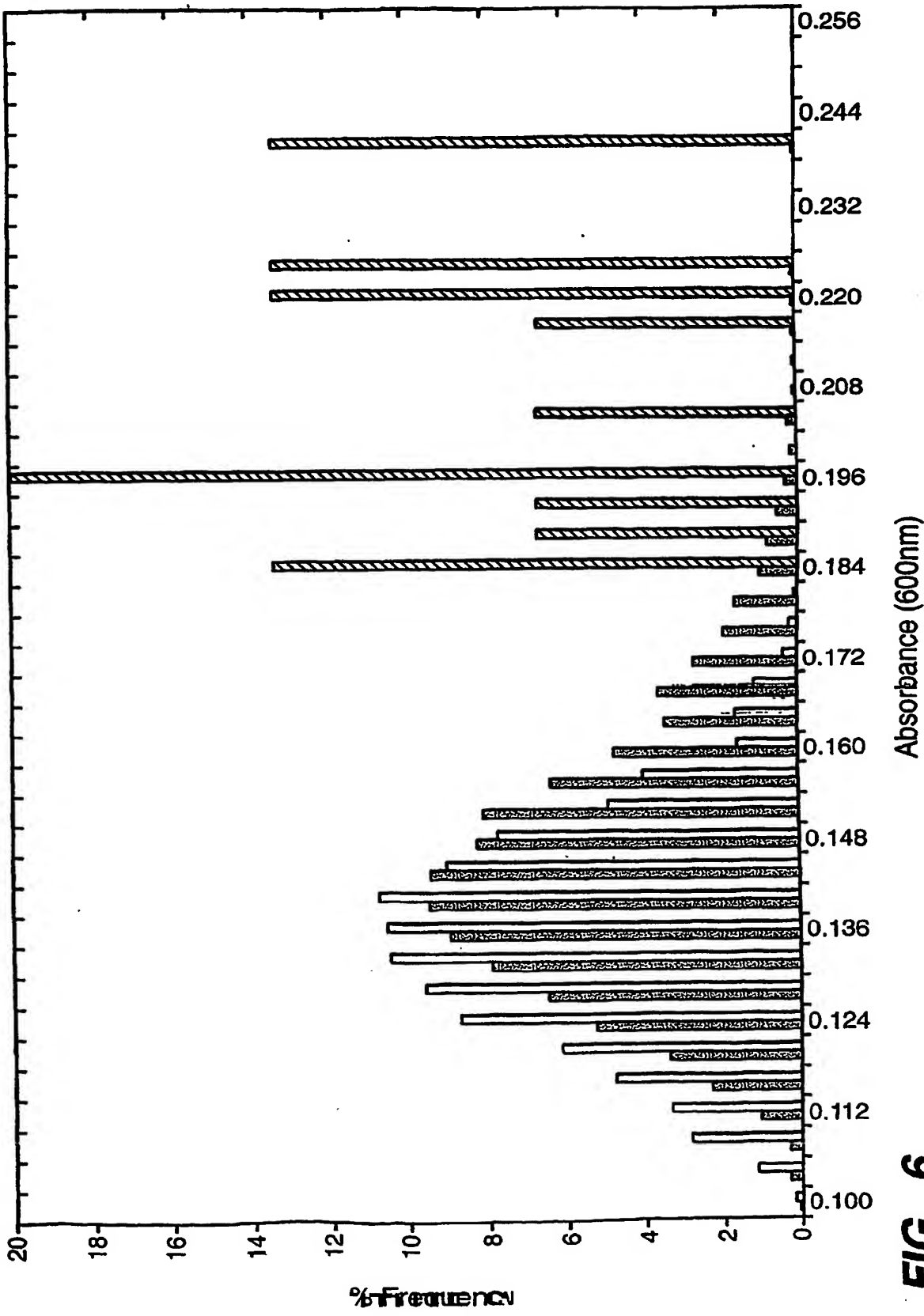
0.256
0.244
0.232
0.220
0.208
0.196
0.184
0.172
0.160
0.148
0.136
0.124
0.112
0.100
Absorbance (600nm)
20
18
16
14
12
10
8
6
4
2
0
%Frequency

FIG._6

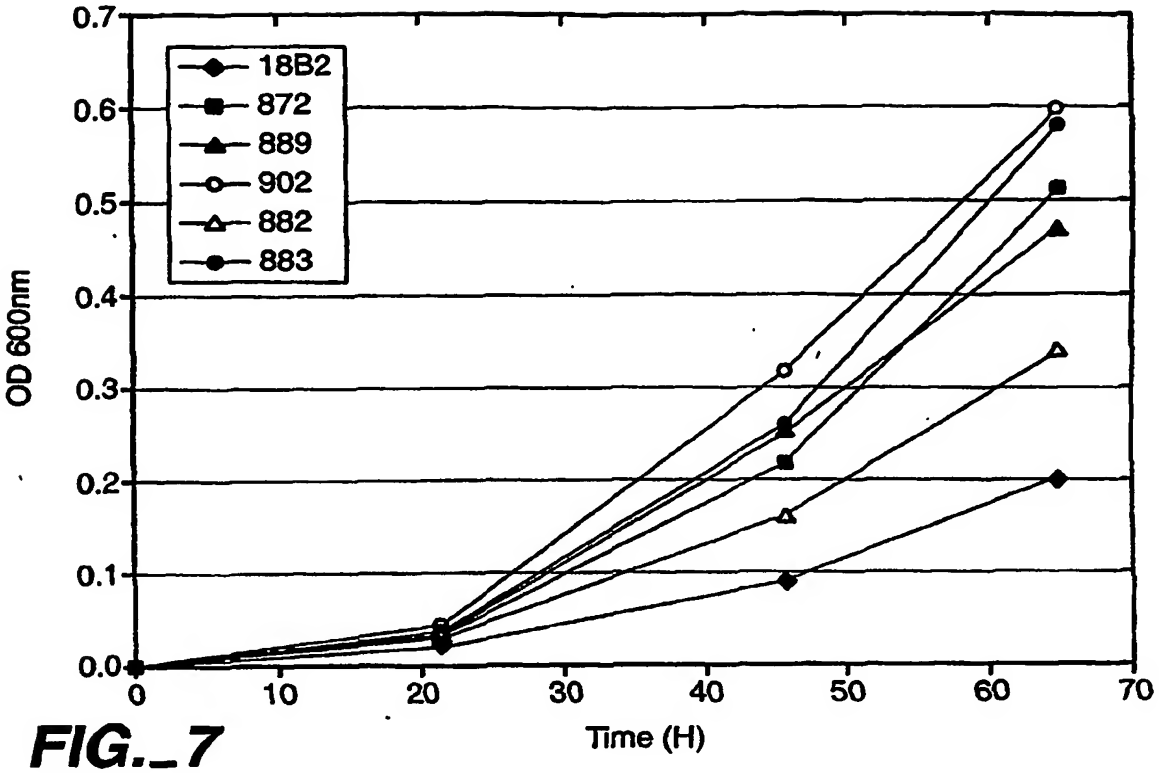
18B2
872
889
902
882
883
OD 600nm
Time (H)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
10
20
30
40
50
60
70

FIG._7

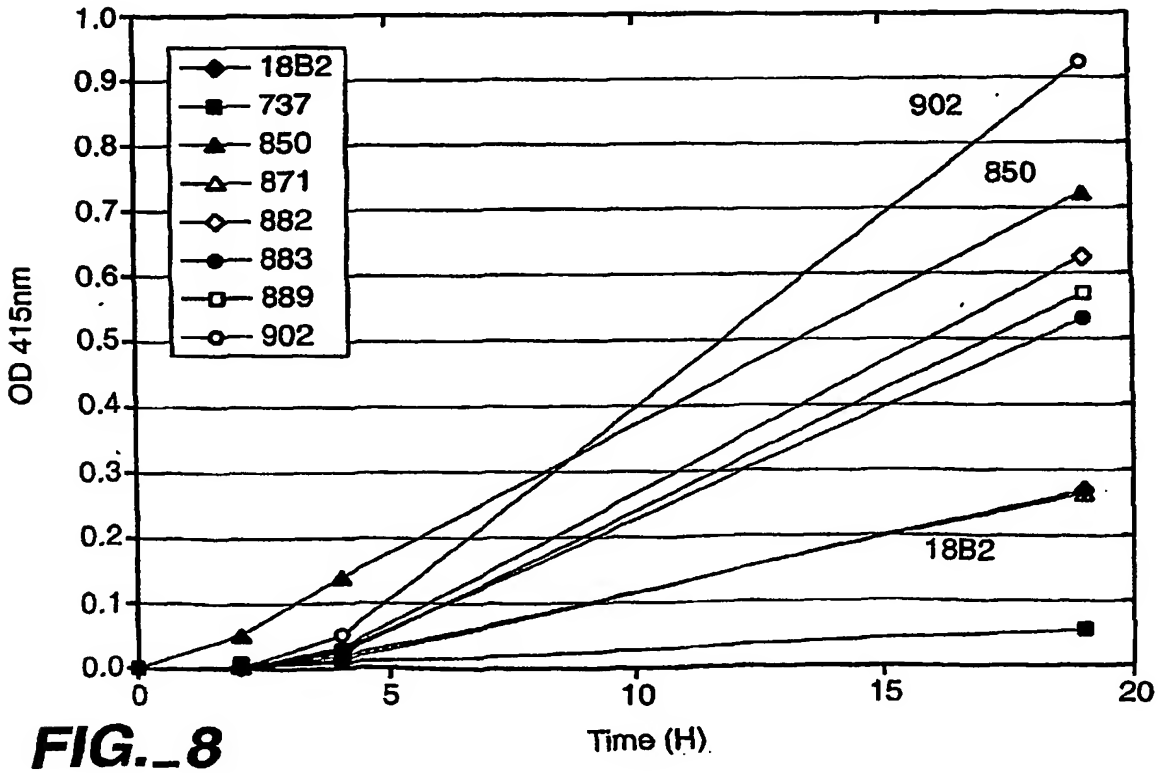
18B2
737
850
871
882
883
889
902
902
850
18B2
OD 415nm
Time (H)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5
10
15
20

FIG._8

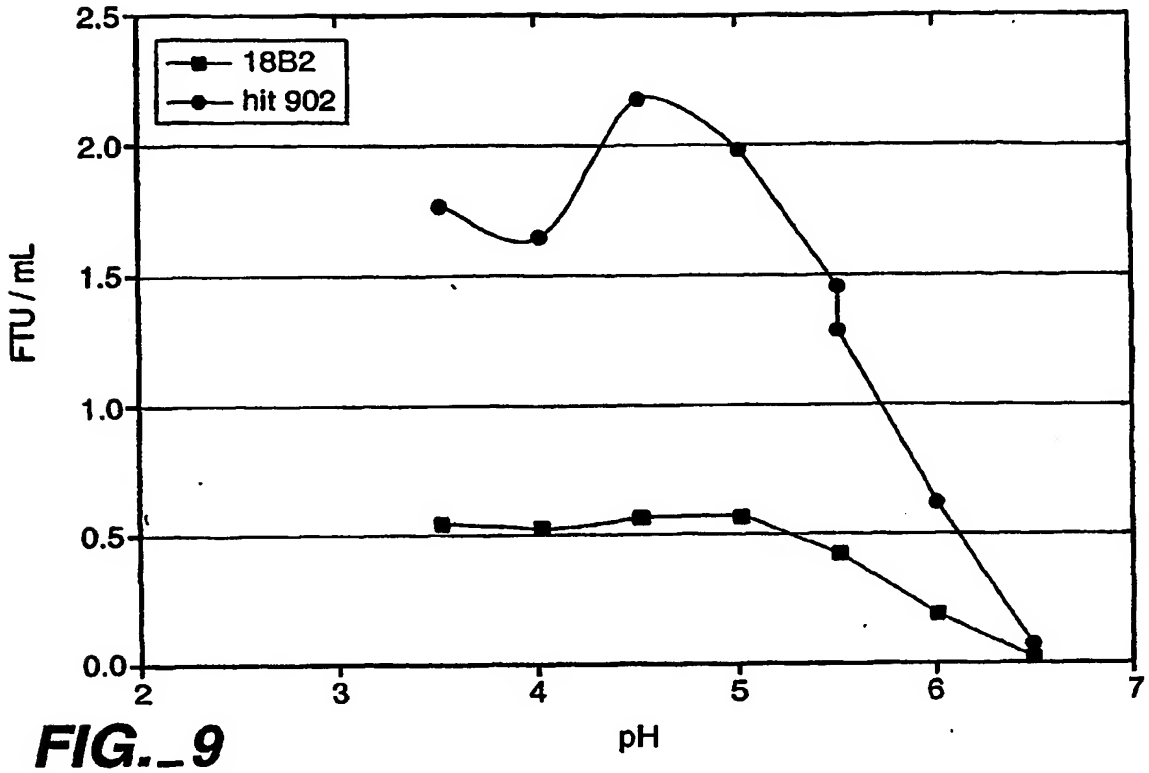
18B2
hit 902
FTU / mL
2.5
2.0
1.5
1.0
0.5
0.0
2
3
4
5
6
7
pH

FIG._9

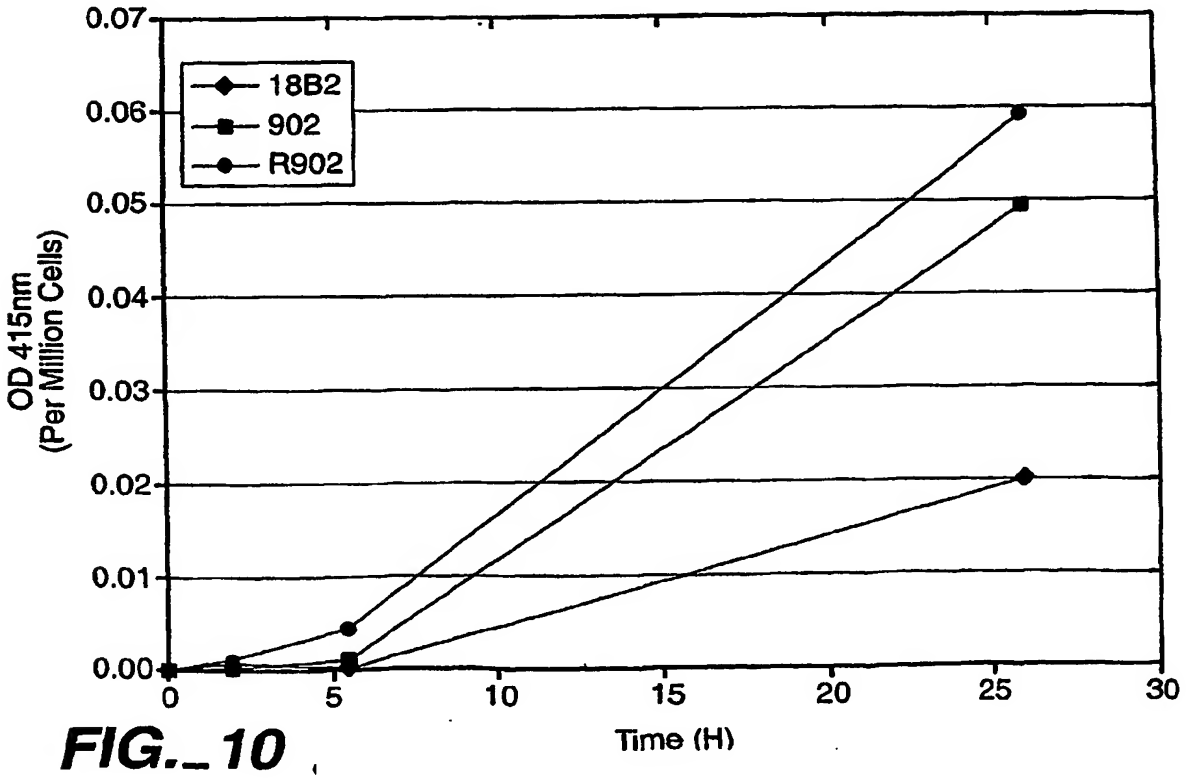
18B2
902
R902
OD 415nm
(Per Million Cells)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0
5
10
15
20
25
30
Time (H)

FIG._10

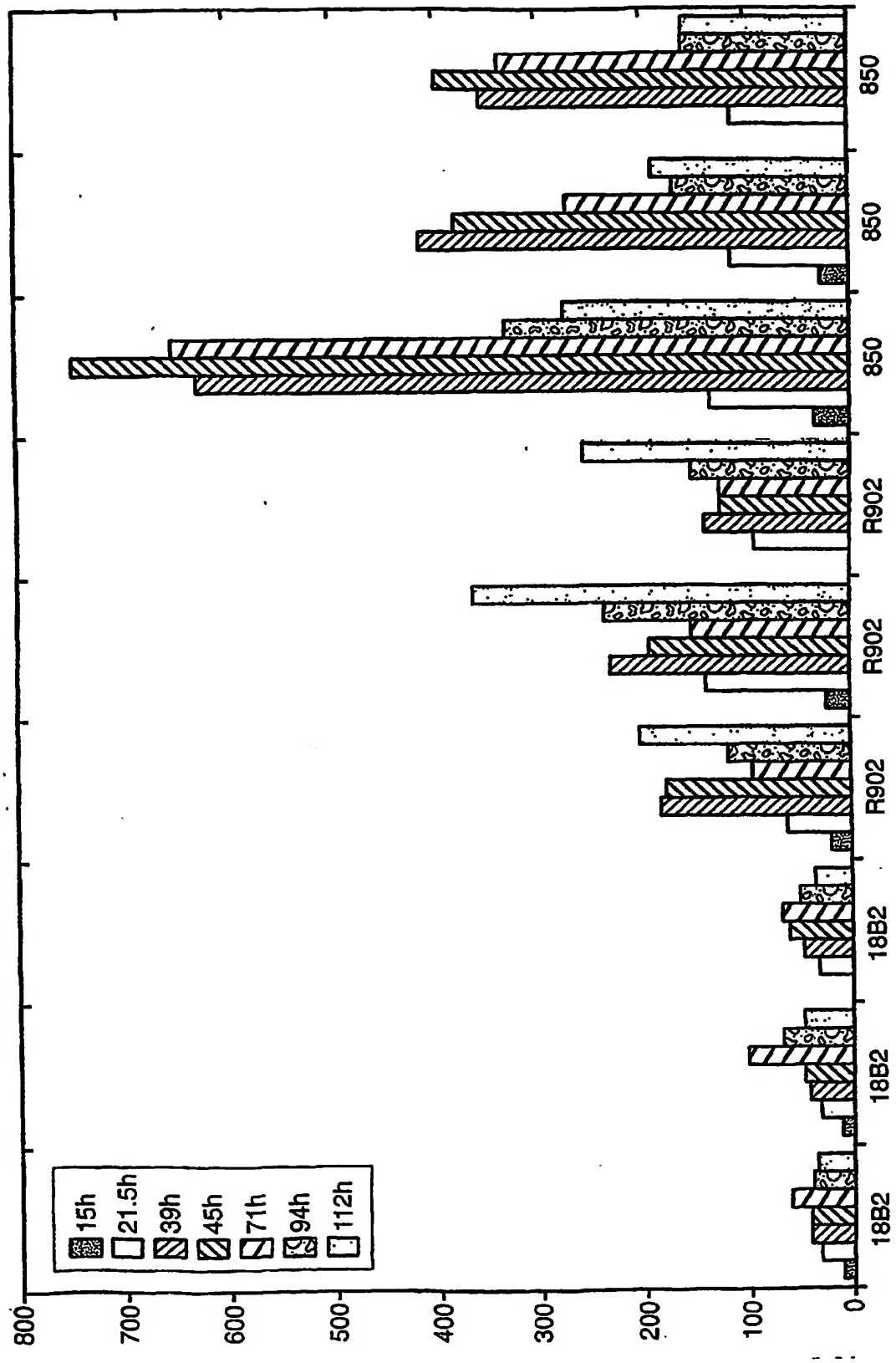
850
850
850
R902
R902
R902
18B2
18B2
18B2
15h
21.5h
39h
45h
71h
94h
112h
800
700
600
500
400
300
200
100
0

FIG._11

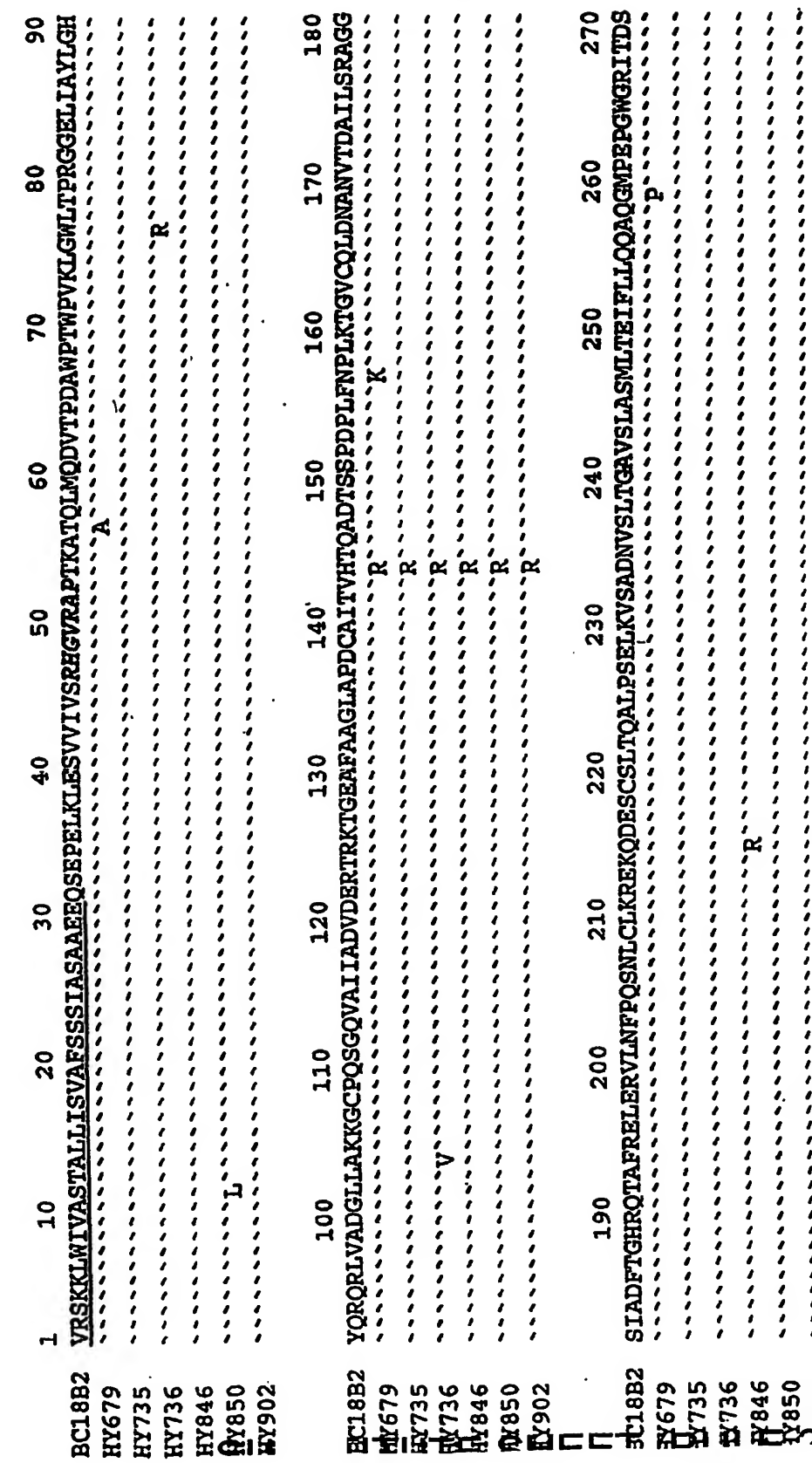
1 10 20 30 40 50 60 70 80 90
BC18B2 VRSKKLWIVASTALLISVAFSSSIASAAEEQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGH
HY679 A
HY735 R
HY736
HY846 L
HY850
HY902
100 110 120 130 140 150 160 170 180
BC18B2 YQRQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDAILSRAGG
HY679 R K
HY735 R
HY736 R V
HY846 R
HY850 R
HY902 R
190 200 210 220 230 240 250 260 270
BC18B2 SIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQAQGMPEPGWGRITDS
HY679 p
HY735
HY736 R
HY846
HY850
HY902

FIG._12A

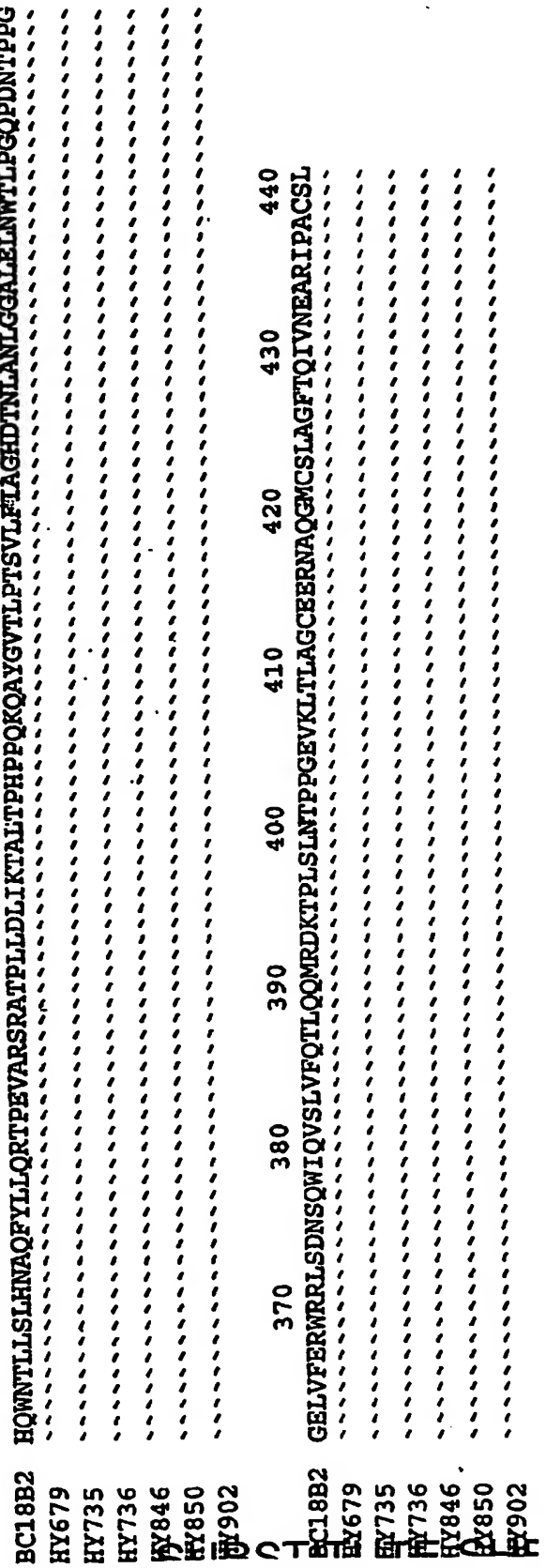
280 290 300 310 320 330 340 350 360
BC18B2 HQWNTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKTALTPHPPQKQAYGVTLPTSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPG
HY679
HY735
HY736
HY846
HY850
HY902
370 380 390 400 410 420 430 440
BC18B2 GELVFERWRRLSDNSQWIQVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSL
HY679
HY735
HY736
HY846
HY850
HY902

FIG._12B

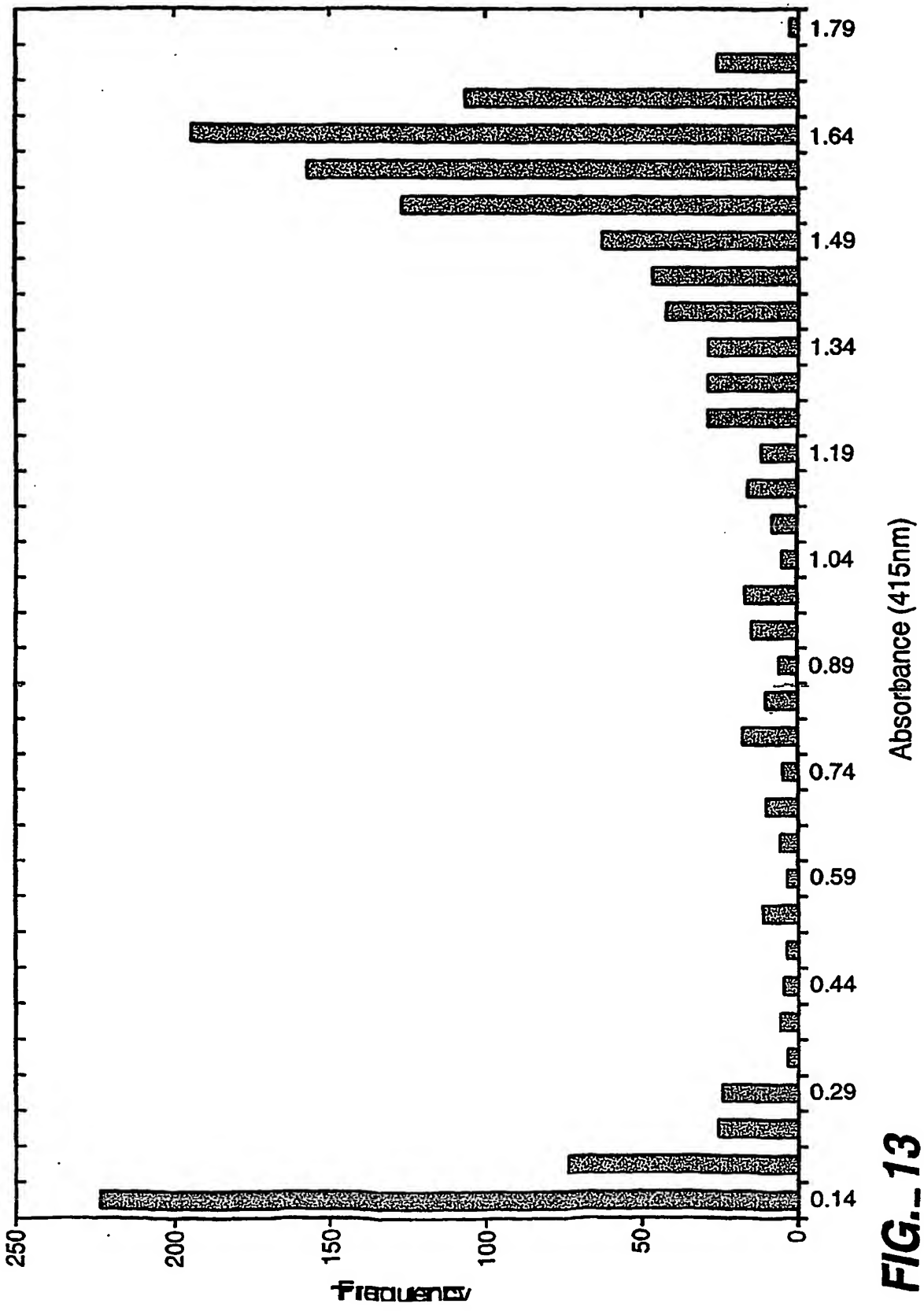
1.79
1.64
1.49
1.34
1.19
1.04
0.89
0.74
0.59
0.44
0.29
0.14
Absorbance (415nm)
250
200
150
100
50
0
Frequency

FIG._13

```
        1       10        20        30        40        50        60        70        80        90
3C18B2  VRSKKLWIVASTALLISVAFSSSIASAAEEQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGH
HY850   ----------L-------------------------------------------------------------E-----------------
HY1361  ----------L------------------------------------------------------------------------V------
HY1363  ----------L-------------------------------------------------------------------------------

               100       110       120       130       140       150       160       170       180
3C18B2  YQRQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDAILSRAGG
HY850   ----------------------------------------------------R-------------------------------------
HY1361  ----------------------------------------------------R-------------------------------------
HY1363  ----------------------------------------------------R-------------------------------------

               190       200       210       220       230       240       250       260       270
3C18B2  SIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDS
HY850   ------------------------------------------------------------------------------------------
HY1361  ------------------------------------------------------------------------------------------
HY1363  ------------------------------------------------------------------------------------------

               280       290       300       310       320       330       340       350       360
3C18B2  HQWNTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKTALTPHPPQKQAYGVTLPTSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPG
HY850   ------------------------------------------------------------------------------------------
HY1361  ------------------------------------------------------------------------------------------
HY1363  ------------------------------------------------------------------------------------------

               370       380       390       400       410       420       430       440
3C18B2  GELVFERWRRLSDNSQWIQVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSL
HY850   -------------------------------------------------------------------------------S
HY1361  -----------------------------------------------------D--------------------------
HY1363  -----------------------------------------------------V--------------------------
```

FIG._14

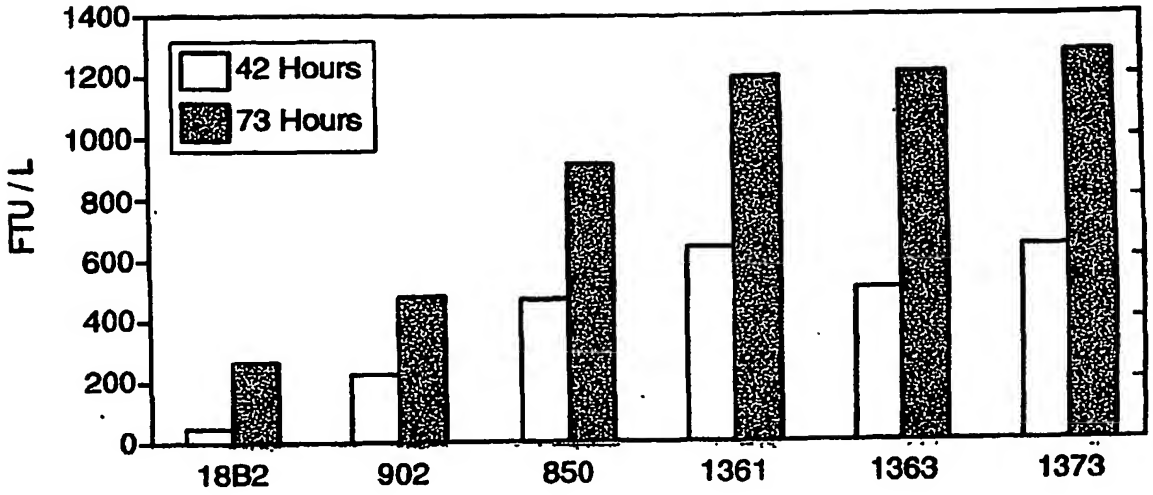
1400
1200
1000
800
600
400
200
0
FTU/L
42 Hours
73 Hours
18B2
902
850
1361
1363
1373

FIG._15

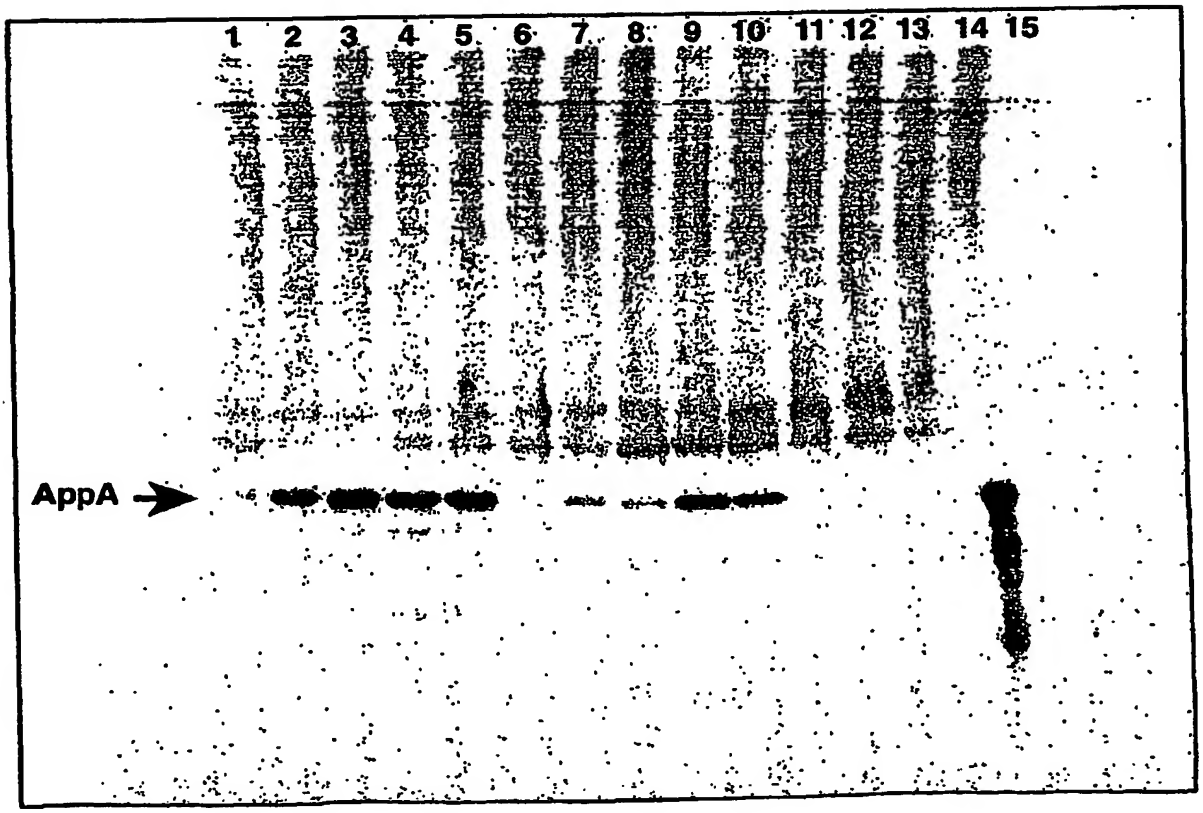
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
AppA

FIG. 17

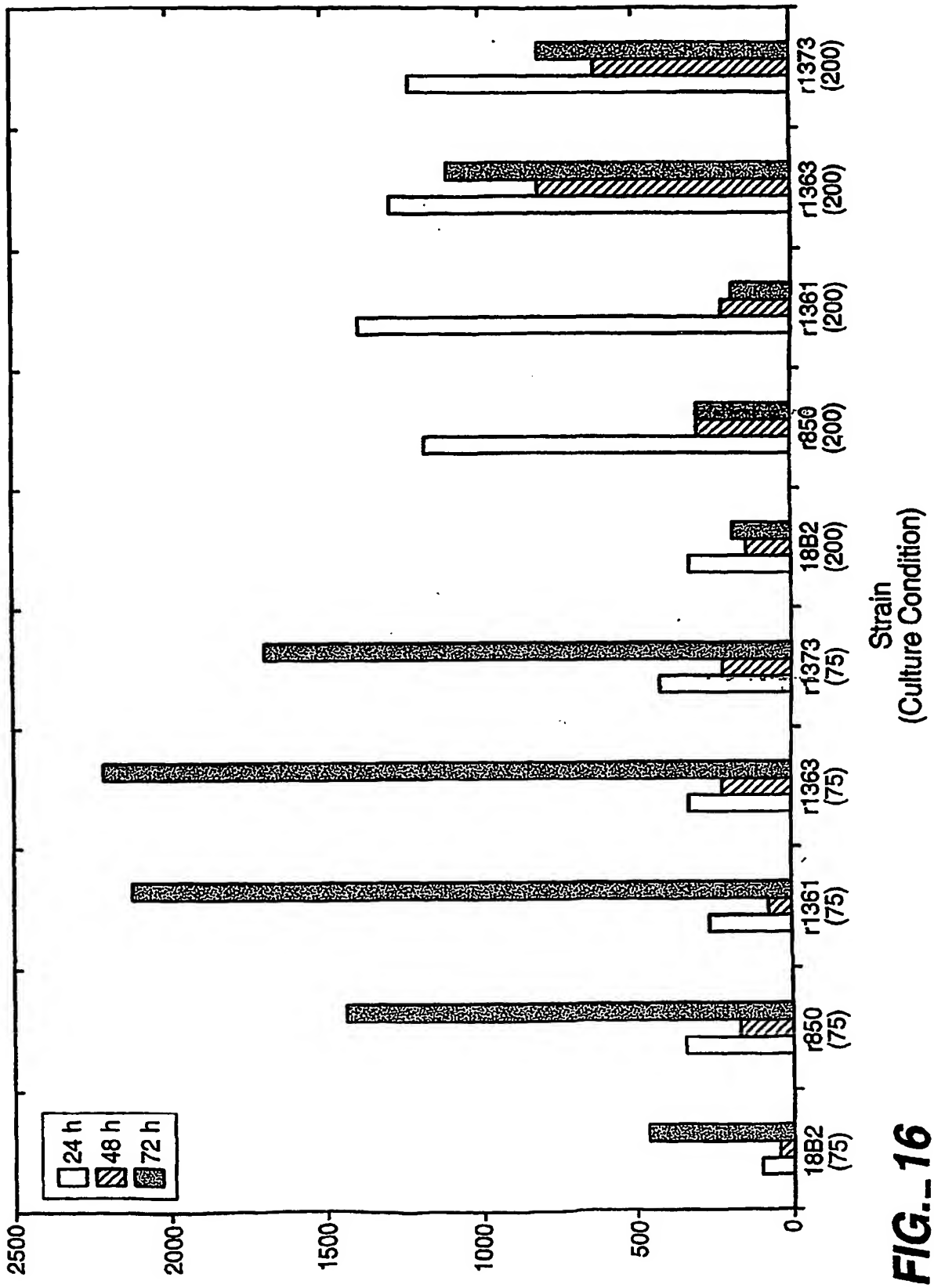
2500
2000
1500
1000
500
0
24 h
48 h
72 h
18B2 (75)
r850 (75)
r1361 (75)
r1363 (75)
r1373 (75)
18B2 (200)
r850 (200)
r1361 (200)
r1363 (200)
r1373 (200)
Strain (Culture Condition)

FIG._16

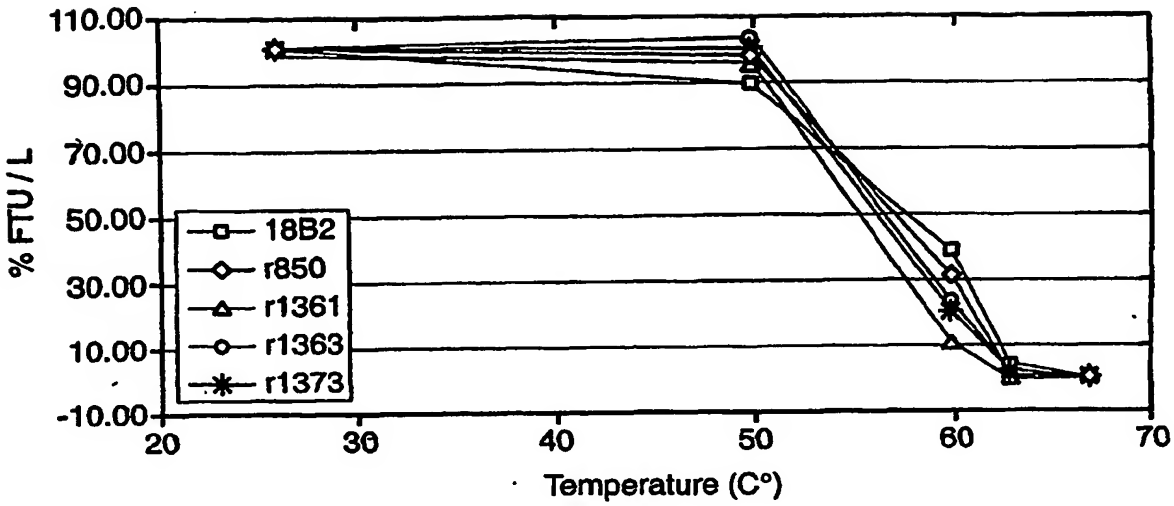

FIG._18

Oligonucleotide primers and combinations thereof used to amplify *AppA* related sequences.

AppA3F 5'-atgaaagcgatcttaat
AppA5F 5'-cgtcatggtgtgcgtgctcc
AppAGF 5'-cgccagaggttgcccg
App/CR 5'-gcggctggcaacctctgg
AppA4R 5'-ttacaaactgcacgccggtatgcgtgcgtgcttcatt

Primer combinations:

AppA 3F+4R = 1.3kb product
AppA 3F+7R = 0.86kb product
AppA 5F+4R = 1.19kb product
AppA 6F+4R = 0.44kb product
AppA SF+7R 0.75kb product

FIG._19

Source	10	20	30	40	50	60	70	80
E. coli	MKAILIPFLS	LLIPLTPQSA	FAQSEPELKL	ESVVIVSRHG	VRAPTKATQL	MQDVTPDAWP	TWPVKLGWLT	PRGGELIAYL
Shigella flexnarii	.....	.....S...	.....	.....	.....	.....	.....	.....F
Shigella sonnei	.....	.....	.....	.....	.....	.....	.....	.....
Pasteurella aerogenes				...	.....	.....	.....	.....
Enterobacter cloacae				...	.....	.....	.....	.....F
Enterobacter agglomerans				...	.....	.....	.....	.....
Proteus vulgaris	.....	.....	.....	.....	.....	.....	.....	.....
Zoo Compost Enrichment				...	.....	.....	.....	.....

Source	90	100	110	120	130	140	150	160
E. coli	GHYQRQRLVA	DGLLAKKGCP	QSGQVAIIAD	VDERTRKTGE	AFAAGLAPDC	AITVHTQADT	SSPDPLFNPL	KTGVCQLDNA
Shigella flexnarii	.....	.....	.P.....	.....	.....	.....	.....	.....
Shigella sonnei	.....	.....	.....	.....	.....	.....	.....	.....
Pasteurella aerogenes	.....	.....	.....	.....	.....	.....	.....	.....
Enterobacter cloacae	.....	.....	.P.....	.....	.....	.....	.....	.....
Enterobacter agglomerans	.....	.....	.P.....	.....	.....	.....	.....	.....
Proteus vulgaris	.....	.....	.....	.....	.....	.....	.....	.....
Zoo Compost Enrichment	.....	.....	.....	.....	.....	.....	.....	.....

Source	170	180	190	200	210	220	230	240
E. coli	NVTDAILSRA	GGSIADFTGH	RQTAFRELER	VLNFPQSNLC	LKREKQDESC	SLTQALPSEL	KVSADNVSLT	GAVSLASMLT
Shigella flexnarii	.....	.....	.....	.....	.....	.....	.....	.....
Shigella sonnei	.....	.....	.....	.....	.....	.....	.....A	.....
Pasteurella aerogenes	.....	.....	.....	.....	.....	.....	.....	.....
Enterobacter cloacae	.....	.....	.....	.....	.....	.....	.....	.....
Enterobacter agglomerans	.....	.....	.....	.....	.....	.....	.....	.....
Proteus vulgaris	.....	.....	.....	.....	F.....	.....	.....	.....
Zoo Compost Enrichment	.....	.....	.....	.....	.....	.....	C	.....

FIG._20A

Source	250	260	270	280	290	300	310	320
E. coli	EIFLLQQAQG	MPEPGWGRIT	DSHQWNTLLS	LHNAQFYLLQ	RTPEVARSRA	TPLLDLIKTA	LTPHPPQKQA	YGVTLPTSVL
Shigella flexnarii	.....	.....	.....	.....	.....	.....MA	.....	.....
Shigella sonnei	.....	.....	.....	.....	.....	.....	.....	.....
Pasteurella aerogenes	.....	.....	.....	.....	.....	.		
Enterobacter cloacae	.....	.....	N.....	.....	.....			
Enterobacter agglomerans	.....	.....	.....	.....	.....			
Proteus vulgaris	.....	.....	.....	I	.....			
No Compost Enrichment	.....	.....	.....S....	.....	.....			

Source	330	340	350	360	370	380	390	400
E. coli	FIAGHDTNLA	NLGGALELNW	TLPGQPDNTP	PGGELVFERW	RRLSDNSQWI	QVSLVFQTLQ	QMRDKTPLSL	NTPPGEVKLT
Shigella flexnarii	.....	.F.....	.....	.....	.....	.....	.....	.....
Shigella sonnei	.....	.....	.....	.....	.....	.....	.....	.....

Source	410	420	430	440
E. coli	LAGCEERNAQ	GMCSLAGFTQ	IVNEARIPAC	SL*
Shigella flexnarii	.....	.....	.....	..
Shigella sonnei	.....	.....	.....	..

FIG._20B

```
        1        *         20        *         40        *         60        *         80
E. coli ATGAAAGCGATCTTAATCCCATTTTTATCTCTTCTGATTCCGTTAACCCCGCAATCTGCATTCGCTCAGAGTGAGCCGGA
S. flex.------------------------------------------------T-------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.--------------------------------------------------------------------------------
compost

                 *        100        *        120        *        140        *        160
E. coli GCTGAAGCTGGAAAGTGTGGTGATTGTCAGTCGTCATGGTGTGCGTGCTCCAACCAAGGCCACGCAACTGATGCAGGATG
S. flex.--------------------------------------------------------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.                              --------------------------------------------------
E. cloa.                              --------------------------------------------------
E. aggl.                              --------------------------------------------------
P. vulg.--------------------------------------------------------------------------------
compost                               --------------------------------------------------

                 *        180        *        200        *        220        *        240
E. coli TCACCCCAGACGCATGGCCAACCTGGCCGGTAAAACTGGGTTGGCTGACACCGCGCGGTGGTGAGCTAATCGCCTATCTC
S. flex.-----------------------------------------------------------------------------T--
S. sonn.--------------------------------------------------------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.-----------------------------------------------------------------------------T--
E. aggl.--------------------------------------------------------------------------------
P. vulg.--------------------------------------------------------------------------------
compost --------------------------------------------------------------------------------
```

FIG._21A

```
                  *       260         *       280         *       300         *       320
E. coli GGACATTACCAACGCCAGCGTCTGGTAGCCGACGGATTGCTGGCGAAAAAGGGCTGCCCGCAGTCTGGTCAGGTCGCGAT
S. flex.--------------------------G-----------------A-----------T------C----------------
S. sonn.--------------------------------------------------------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.--------------------------G-----------------A-----------T------C----------------
E. aggl.--------------------------------------------A-----------T------C----------------
P. vulg.--------------------------------------------------------------------------------
compost --------------------------------------------------------------------------------

                  *       340         *       360         *       380         *       400
E. coli TATTGCTGATGTCGACGAGCGTACCCGTAAAACAGGCGAAGCCTTCGCCGCCGGGCTGGCACCTGACTGTGCAATAACCG
S. flex.--------------------------------------------------------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.--------------------------------------------------------------------------------
E. aggl.--------------------------------------------------------------------------------
P. vulg.--------------------------------------------------------------------------------
compost --------------------------------------------------------------------------------

                  *       420         *       440         *       460         *       480
E. coli TACATACCCAGGCAGATACGTCCAGTCCCGATCCGTTATTTAATCCTCTAAAAACTGGCGTTTGCCAACTGGATAACGCG
S. flex.----------------------------------------------------------------------------T---
S. sonn.-------------T------------------------------------------------------------------
P. aero.-------------T------------------------------------------------------------------
E. cloa.----------------------------------------------------------------------------T---
E. aggl.----------------------------------------------------------------------------T---
P. vulg.-------------T------------------------------------------------------------------
compost --------------------------------------------------------------------------------
```

FIG._21B

10/524234


```
                    *       500         *       520         *       540         *       560
E. coli AACGTGACTGACGCGATCCTCAGCAGGGCAGGAGGGTCAATTGCTGACTTTACCGGGCATCGGCAAACGGCGTTTCGCGA
S. flex.--------------------------------------------------------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.--------------------------------------------------------------------------------
E. aggl.--------------------------------------------------------------------------------
P. vulg.--------------------------------------------------------------------------------
compost --------------------------------------------------------------------------------

                    *       580         *       600         *       620         *       640
E. coli ACTGGAACGGGTGCTTAATTTTCCGCAATCAAACTTGTGCCTTAAACGTGAGAAACAGGACGAAAGCTGTTCATTAACGC
S. flex.--------------------------------------------------------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.--------------------------------------------------------------------------------
E. aggl.--------------------------------------------------------------------------------
P. vulg.----------------------------------------T---------------------------------------
compos. --------------------------------------------------------------------------------

                    *       660         *       680         *       700         *       720
E. coli AGGCATTACCATCGGAACTCAAGGTGAGCGCCGACAATGTCTCATTAACCGGTGCGGTAAGCCTCGCATCAATGCTGACG
S. flex.--------------------------------------------------------------------------------
S. sonn.-----------------------------------------------G--------------------------------
P. aero.--------------------------------------------------------------------------------
E. cloa.--------------------------------------------------------------------------------
E. aggl.--------------------------------------------------------------------------------
P. vulg.--------------------------------------------------------------------------------
compost --------------------------------------------C-----------------------------------
```

FIG._21C

```
                      *       740         *       760         *       780         *       800
E. coli  GAGATATTTCTCCTGCAACAAGCACAGGGAATGCCGGAGCCGGGGTGGGGAAGGATCACCGATTCACACCAGTGGAACAC
S. flex. -----------------------------------A--------------------------------------------
S. sonn. --------------------------------------------------------------------------------
P. aero. --------------------------------------------------------------------------------
E. cloa. -----------------------------------A-----------------------A--------------------
E. aggl. -----------------------------------A--------------------------------------------
P. vulg. --------------------------------------------------------------------------------
compost  ----------------------------------------------------------------------------G---

                      *       820         *       840         *       860         *       880
E. coli  CTTGCTAAGTTTGCATAACGCGCAATTTTATTTGCTACAACGCACGCCAGAGGTTGCCCGCAGCCGCGCCACCCCGTTAT
S. flex. --------------------------------------------------------------------------------
S. sonn. --------------------------------------------------------------------------------
P. aero. ----------------------------------
E. cloa. -------------------------------------------------------------------
E. aggl. -------------------------------------------------------------------
P. vulg. ----------------------------------A---------------------------------
compost  ----------------------------------------------------------------

                      *       900         *       920         *       940         *       960
E. coli  TAGATTTGATCAAGACAGCGTTGACGCCCCATCCACCGCAAAAACAGGCGTATGGTGTGACATTACCCACTTCAGTGCTG
S. flex. -G---------T-G--------------------------------------------------------------A---
S. sonn. --------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost
```

FIG._21D

```
                     *        980         *        1000         *        1020         *        1040
E. coli  TTTATCGCCGGACACGATACTAATCTGGCAAATCTCGGCGGCGCACTGGAGCTCAACTGGACGCTTCCCGGTCAGCCGGA
S. flex. -----T---------------------------T----------------------------------------------
S. sonn. --------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost

                     *       1060         *        1080         *        1100         *        1120
E. coli  TAACACGCCGCCAGGTGGTGAACTGGTGTTTGAACGCTGGCGTCGGCTAAGCGATAACAGCCAGTGGATTCAGGTTTCGC
S. flex. --------------------------------------------------------------------------------
S. sonn. --------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost

                     *       1140         *        1160         *        1180         *        1200
E. coli  TGGTCTTCCAGACTTTACAGCAGATGCGTGATAAAACGCCGCTGTCATTAAATACGCCGCCCGGAGAGGTGAAACTGACC
S. flex. --------------------------------------------------------------------------------
S. sonn. --------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost
```

FIG._21E

```
                 *     1220  .    *     1240      *     1260      *     1280
E. coli CTGGCAGGATGTGAAGAGCGAAATGCGCAGGGCATGTGTTCGTTGGCAGGTTTTACGCAAATCGTGAATGAAGCACGCAT
S. flex.--------------------------------------------------------------------------------
S. sonn.--------------------------------------------------------------------------------
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost

                   1309
E. coli ACCGGCGTGCAGTTTGTAA
S. flex.----------------^^^
S. sonn.----------------^^^
P. aero.
E. cloa.
E. aggl.
P. vulg.
compost
```

FIG._21F